Microbiological profile of whey with or without inclusion of a bacterial inoculant at different cooling times for use as silage additive

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Introduction Tropical forage plants used for ensilage have unfavorable fermentative characteristics in their growth stages with high nutritional value, threatening the preservation process owing to undesirable secondary fermentations. The use of additives in silages in order to improve the fermentation pattern and increase the energy available for the development and multiplication of lactic acid bacteria has been an excellent method of overcoming this limitation. In this context, the objective was to evaluate the microbiological profile of whey with or without the inclusion of a bacterial inoculant at different cooling times.

Materials and Methods The experiment was carried out at the State University of West Paraná - UNIOESTE, Campus of Marechal Cândido Rondon, Paraná. Liquid whey samples were collected at a Whey Powder Production Unit located in the municipality. Whey was collected shortly after its arrival at the unit and immediately refrigerated. The samples either received or did not receive a bacterial inoculant (composition: *Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus acidophilus, Pediococcus acidilactici, Enterococcus faecium, Lactobacillus buchneri, Lactococcus lactis,* and *Propionibacterium acidipropionici* at concentrations of 1.0×10^{10} CFU g^{-1}) according to the manufacturer's recommendations. The samples were refrigerated in hermetically sealed glasses at a temperature of 3°C for microbiological quantification at 0, 24, 48, and 72 hours after application of the inoculant. Bacterial populations were determined by culture techniques according to Silva *et al*. (2007), using the following media: Lactobacillus MRS Broth for counting lactic acid bacteria (LAB), incubated at 30°C for 48 hours; Violet Red Bile Agar for counting enterobacteria, incubated at 36°C for 24 hours; Reinforced Clostridial Agar for counting *Clostridium*, incubated for 24 hours in a greenhouse with a carbon gas system at 36°C; and Plate Count Agar for counting mesophilic aerobic bacteria (MAB), incubated at 35°C for 48 hours. After the incubation period, colonies were counted using a Quebec colony counter, and the plaques that presented between 30 and 300 CFUs (Colony Forming Units) per Petri dish were counted. The results were expressed as the log of CFU g^{-1}. The data were analyzed in a completely randomized design with repeated measures over time. A mixed model was used with fixed effects of the inoculant (1GL), cooling time (3GL), and its interactions (3GL), and the random effect of the experimental error (18GL) using the MIXED procedure of SAS® University Edition. Among all investigated error structures, the first-order AR (1) autoregressive structure was the best according to the Bayesian information criterion (BIC). In all analyses, significance was declared at $P \leq 0.05$.

Results and Discussion The populations of *Clostridium*, enterobacteria, LAB, and mesophilic aerobic bacteria differed ($P < 0.05$) between cooling times of the whey with or without addition of the commercial inoculant (Table 1). A larger undesirable bacterial population was observed

with increased refrigeration period, showing that refrigeration at 3°C is insufficient to prevent the growth of *Clostridium*, enterobacteria, and MAB. With the use of the inoculant, the *Clostridium* population size increased with refrigeration time, showing that the inoculant was not sufficient to prevent *Clostridium* multiplication. The development of enterobacteria was not affected (P > 0.05) by the treatments, whereas the growth of *Clostridium* and MAB was higher in the whey with addition of the commercial inoculant after 24, 48, and 72 hours of refrigeration. The use of additives in silages containing high populations of enterobacteria and *Clostridium* produces undesirable secondary fermentations and causes degradation of proteins, impairing the preservation process and reducing the acceptability of roughage for the animals. Meanwhile, the inoculation of silages with microbial additives based on LAB promotes fermentation of soluble carbohydrates, producing acids that reduce the pH of the ensiled mass. This improves preservation, minimizing losses and reducing the growth of undesirable microorganisms that compete for nutrients. The LAB population increased with cooling time both with or without the use of the inoculant. The LAB population was larger with the inoculant than without it only at the 48-hour time point.

Table 1 Bacterial population (log CFU g^{-1}) present in whey with or without inclusion of a bacterial inoculant at different cooling times

	Clostridium				Enterobacteria			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
Without inoculant	5.10Ca	5.34Cb	6.51Bb	7.23Ab	4.35Ca	4.50Ca	5.85Ba	6.55Aa
With inoculant	5.15Da	6.23Ca	7.39Ba	8.23Aa	4.14Da	5.18Ca	6.80Ba	6.03Aa
SEM								
Inoculant			0.07				0.23	
Cooling Time			0.09				0.22	
Inoculant*Cooling Time			0.13				0.31	
	Lactic acid bacteria				Mesophilic aerobic bacteria			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
Without inoculant	5.20Ca	5.36BCa	5.75Bb	7.04Aa	5.28Ca	5.22Cb	6.25Bb	6.97Ab
With inoculant	5.20Da	5.69Ca	6.50Ba	7.37Aa	5.12Da	5.87Ca	6.96Ba	7.97Aa
SEM								
Inoculant			0.07				0.10	
Cooling Time			0.10				0.13	
Inoculant*Cooling Time			0.14				0.18	

Means followed by the same letter, lowercase in the columns and uppercase in the row, do not differ by Tukey's test (P <0.05). SEM: Standard error of the mean.

Conclusions Whey is a source of LAB for use as an inoculant in silage; however, it should not be stored for more than 24 hours. The inclusion of an inoculant in the whey increases the population of *Clostridium* and mesophilic aerobic bacteria when the whey is stored under refrigeration.

References

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